Title
MinK potassium channels are heteromultimeric complexes.

Journal
The Journal of biological chemistry, 272(3)

ISSN
0021-9258

Authors
Tai, KK
Wang, KW
Goldstein, SA

Publication Date
1997

DOI
10.1074/jbc.272.3.1654

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 272, No. 3, Issue of January 17, pp. 1654–1658, 1997

Printed in U.S.A.

MinK Potassium Channels Are Heteromultimeric Complexes*

(Received for publication, October 23, 1996, and in revised form, November 14, 1996)

Kwok-Keung Tai, Ke-Wei Wang, and Steve A. N. Goldstein‡

From the Departments of Pediatrics and Cellular and Molecular Physiology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536-0812

MinK is a transmembrane protein of 130 amino acids found in the kidney, heart, and vestibular system of mammals. Its expression in *Xenopus laevis* oocytes induces a voltage-dependent potassium current similar to that seen *in vivo*. Indirect evidence has fueled speculation that function requires association of MinK and another protein endogenous to oocytes and native tissues. In this report, we show that direct covalent modification of an oocyte membrane protein alters properties of the MinK ion conduction pore; modified channels exhibit decreased potassium conduction and increased permeability to sodium and cesium. The modifying reagents, two membrane-impermeant, sulfhydryl-specific methanethiosulfonate derivatives, react only from the extracellular solution at rates that are determined by the conformational state of the channel. These findings indicate that MinK is intimately associated with an oocyte protein whose exposure to the external solution changes during channel gating and which acts with MinK to establish ion conduction pore function.

Ion channel proteins form transmembrane aqueous pores that open and close in response to specific stimuli and thereby mediate cellular electrical activity. Voltage-gated K^+ channels govern the activity of cyclically excitable tissues (like nerves and muscles) by acting to repolarize cells after stimulation. Originally isolated from rat kidney based on its ability to induce voltage-activated K^+ currents in *Xenopus* oocytes (1), MinK enjoys wide tissue distribution in mammals (2). In oocytes, MinK currents exhibit many characteristics of classical voltage-dependent, K^+-selective channels (3, 4) and show notable similarities to the delayed rectifier K^+ currents present in cardiac myocytes (2, 5–7) and vestibular dark cells (8); this has led to the hypothesis that MinK underlies these currents *in vivo*.

Because MinK bears no sequence homology to other K^+ channel proteins, uncertainty has surrounded its role in channel function. Early confusion that MinK could regulate Cl^- channels (9) has proven to be an artifact of over-expression (10, 11) and given way to the sentiment that MinK is a structural protein intrinsic to channel formation (4). Thus, point mutations have identified MinK residues that modify almost all channel functions including activation by voltage (12, 13), regulation by protein kinase C (14, 15), ionic selectivity among monovalent cations (4), and sensitivity to inhibition by open channel pore blockers (4, 16). Recently, we showed that a domain of MinK lines the external portion of the ion conduction pore (16). When mutated to cysteine, sites in this domain render channels susceptible to covalent block by methanethiosulfonate ethylsulfonate ($MTSES^-$)[1] and alter its reversible inhibition by tetraethylammonium (TEA). That TEA blockade is sensitive to the concentration of permeant ions inside the cell (as well as transmembrane voltage) and that $MTSES^-$ and TEA compete for overlapping MinK binding sites argue that both bind to the external end of the MinK conduction pore, ~15% of the way through the potential drop across the membrane.

The subunit composition of MinK channels has also been a matter of controversy (13, 17). With the exception of MinK, cloned K^+ channel subunits possess one or two highly conserved, pore-forming P domains (18, 19), while voltage-gated Ca^{2+} and Na^+ channel subunits contain four P domains (20). In those channels, the ion conduction pathway is formed by four P domains supplied by four, two, or one subunit, respectively (21).[2] MinK has no P domains by which to predict its subunit stoichiometry. We have supported a heteromultimeric model containing two MinK monomers, based on the effects of coexpression of MinK and MinK mutants (13), and a required non-MinK subunit that oocytes provide but only in a limited amount since isochronal currents saturate despite increasing levels of MinK protein on the oocyte surface (13, 16). A role for a non-MinK subunit has also been proposed by Blumenthal and Kaczmarek (22) based on similar findings and by Lesage *et al.* (23) based on their study of three cell lines that fail to show currents despite MinK surface expression. Others have speculated that MinK may associate with HERG K^+ channel subunits based on inhibition of I_{Kr} currents in a cardiac cell line treated with MinK antisense oligonucleotides (24).

In this report, we show that K^+ channels induced in oocytes by a cysteine-free mutant of MinK are sensitive to two sulfhydryl-specific reagents that covalently modify free cysteine residues exposed to aqueous solvent. Modification alters MinK ion conduction and ion selectivity, two fundamental properties of the channel pore. Channels are sensitive to modification only from the extracellular solution, and the rate of reaction depends on the channel conformational state. These results indicate that a cysteine-bearing protein endogenous to oocytes is intimately associated with the channel gating and conduction apparatus and suggest it may contribute directly to the MinK pore.

EXPERIMENTAL PROCEDURES

Mutants of rat MinK were made in pSD (16) and cRNA (2 ng) injected into oocytes. Whole cell currents were measured after 2 days by a

* This work was supported by grants from the National Institutes of Health and the Donaghue Foundation (to S. A. N. G.) and from the American Heart Association (to K. W. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Pediatrics and Cellular and Molecular Physiology, Boyer Center for Molecular Medicine, Yale University School of Medicine, 295 Congress Ave., New Haven, CT 06536-0812. Tel.: 203-737-2214; Fax: 203-737-2290; E-mail: steve.goldstein@Yale.Edu.

[1] The abbreviations used are: $MTSES^-$, methanethiosulfonate ethylsulfonate; $MTSEA^+$, MTS-ethylammonium; $MTSET^+$, MTS-ethyltrimethylammonium; TEA, tetraethylammonium; $NMDG^+$, *N*-methyl-D-glucamine.

[2] K. W. Wang and S. A. N. Goldstein, unpublished data.

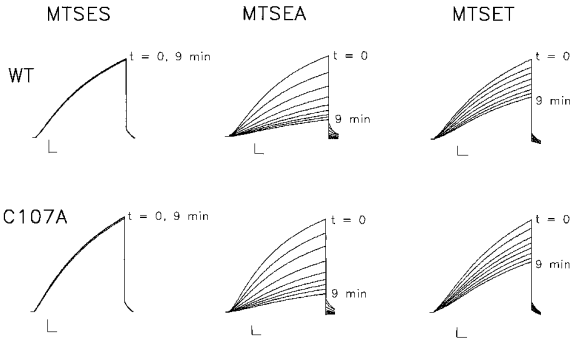

FIG. 1. **Effect of $MTSES^-$, $MTSEA^+$, and $MTSET^+$ on wild-type and C107A-MinK currents.** Whole oocyte currents were recorded by two-electrode voltage clamp before and after the exposure to 10 mM $MTSES^-$, 2.5 mM $MTSEA^+$, or 5 mM $MTSET^+$ for 9 min. Current traces were elicited by 10-s pulses from a holding potential of −80 mV to 0 mV with an interpulse interval of 10 s and are shown after leak subtraction. *Scale bars* represent 100 nA and 1 s.

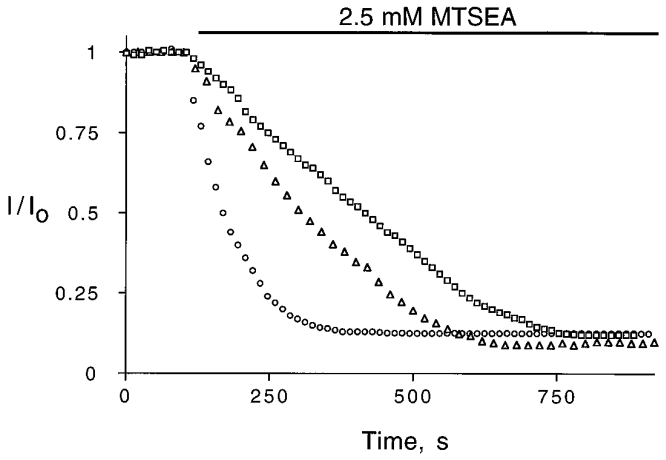

FIG. 2. **Effect of channel state on block kinetics by 2.5 mM $MTSEA^+$.** Each point represents the whole cell current amplitude at the end of the test pulse in an oocyte-expressing C107A-MinK elicited by a test pulse to 0 mV from a holding potential of −80 mV. *Circles* (○) represent currents measured with a test pulse of 3 s and an interpulse interval of 10 s; *triangles* (△), a test pulse of 10 s and an interpulse interval of 10 s; and *boxes* (□), a test pulse of 10 s and an interpulse interval of 3 s. Time constants of block, τ, were determined by single exponential fits to the inhibition time course. The figure shows the results from three individual oocytes; mean values ± S.E. for 5–8 oocytes are reported in the text.

two-electrode voltage clamp with constant perfusion at 22 °C (13). Data were filtered at 100 Hz, sampled at 1 kHz, and leak correction was performed off-line (3, 4). Bath solution was ND-96 (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$, 0.3 $CaCl_2$, and 5 HEPES, pH 7.6) or solutions in which NaCl and KCl were replaced by 98 mM test cation as chloride salt. MTS solutions were made from powder (Toronto Research, Ontario) by substitution for NaCl in ND-96 and used within 15 min. MTS derivatives hydrolyze in aqueous solution (16, 25). The half-life in ND-96 at 22 °C for 5 mM $MTSES^-$ was ~55 min; for 2.5 mM $MTSEA^+$, it was ~ 40 min; and for 10 mM $MTSET^+$, it was ~20 min; hydrolyzed 2.5 mM $MTSEA^+$ was prepared by incubation at 22 °C overnight.

RESULTS

External $MTSEA^+$ and $MTSET^+$ Block Cysteine-free MinK Channels—A panel of water-soluble, membrane-impermeant, thiol-specific methanethiosulfonate (MTS) derivatives developed by Akabas, Karlin and co-workers (25, 26) has gained wide use for evaluation of sites in membrane proteins exposed to aqueous solution (27). These reagents are over 2,500 times more soluble in water than *n*-octanol and form mixed disulfides with free sulfhydryls accessible to aqueous solvent; in proteins, a covalent bond forms between a portion of the reagent ($-SCH_2CH_2X$) and exposed cysteine side chains. In previous work, we found that a negatively charged MTS derivative, $MTSES^-$, did not block wild-type MinK channels in oocytes (Fig. 1) but did block MinK mutants bearing cysteine at positions 44, 45, or 47 (16).

On the other hand, wild-type MinK is blocked by two positively charged MTS derivatives, MTS-ethylammonium ($MTSEA^+$) and MTS-ethyltrimethylammonium ($MTSET^+$) (Fig. 1). The single native cysteine in MinK at position 107 is thought to be intracellular and protected from reagents in the external solution. As expected, mutation of this cysteine to alanine (C107A-MinK) does not relieve block by the two cationic MTS reagents (Fig. 1). This suggests that a non-MinK, cysteine-bearing protein is modified, rather than MinK, to alter channel function. MTS derivatives show marked specificity for free sulfhydryls (25, 28) and alter ion conduction through both anion- and cation-selective channels with free cysteines in their pores (16, 26, 29–32). Thus, a simple model to explain block of MinK by $MTSEA^+$ and $MTSET^+$ is that a protein native to oocytes associates with MinK and exposes a free cysteine close to the MinK channel pore. We sought to support or refute this idea by studying in detail the effects of $MTSEA^+$ on C107A-MinK.

Block of C107A-MinK by $MTSEA^+$ exhibits characteristics expected for covalent modification, it is not reversed upon washout and does not occur with hydrolyzed reagent. Exposure of oocytes expressing C107A-MinK to 2.5 mM $MTSEA^+$ for 5 min or more decreases currents by 87 ± 5% (mean ± S.E., 17 oocytes). Higher concentrations of $MTSEA^+$ (up to 25 mM), longer periods of exposure (up to 20 min), and washout periods up to 30 min do not alter the magnitude of irreversible blockade. This type of stable partial blockade is commonly observed after treatment of channels with $MTSEA^+$ (16, 26, 29–32) and has been shown to reflect a decrease in single-channel conductance in two cases (31, 32). Only weak reversible inhibition is observed when C107A-MinK expressing oocytes are treated with 2.5 mM hydrolyzed $MTSEA^+$, 5 ± 2% (mean ± S.E., 6 oocytes). Inhibition by $MTSEA^+$ is unexpectedly specific; irreversible blockade is not observed when C107A-MinK is treated to a panoply of thiol-specific reagents (for 8 min or more) including 30 mM iodoacetic acid, 30 mM iodoacetamide, 1 mM 3-(*N*-maleimidopropionyl)-biocytin, 1 mM *N*-ethylmaleimide, 1 mM 1-biotinamido-4-(4′-[maleimidomethyl]cyclohexane-carboxamido)butane, 0.4 mM *N*-[6-(biotinamido)hexyl]-3′-(2′-pyridyldithio)propionamide, 2 mM fluorescein mercuric acetate, or 50 mM thimerosal.

That both $MTSEA^+$ and $MTSET^+$ (with a fixed positive charge) block irreversibly from the extracellular solution suggests the modification site is accessible to the bath solution and not buried in a hydrophobic portion of the channel or membrane. This is supported by the observation that intracellular microinjection of 23 nl of 50 mM $MTSEA^+$, yielding an intracellular concentration of ~2.5 mM $MTSEA^+$, produces a current reduction of only 6 ± 5% (mean ± S.E., 4 oocytes measured at 10 and 30 min after injection).

$MTSEA^+$ Block of C107A-MinK Is Faster When Channels Are Closed—The kinetics of MinK blockade by MTS derivatives can be studied by abrupt exposure of oocytes expressing channels to the reagents (16). To test whether $MTSEA^+$ modification is sensitive to channel conformation, we measured the rate of block of C107A-MinK currents with duty cycles of varying durations at the test voltage (0 mV) and holding voltage (−80 mV) (Fig. 2). When oocytes are exposed to 2.5 mM $MTSEA^+$ during cycles with 3-s test pulses and 10-s holding intervals, blocking kinetics follow a roughly exponential relaxation with an apparent time constant (τ) of 112 ± 12 s (mean ± S.E., 8 oocytes). When the fraction of time spent at the holding voltage

is decreased, by increasing the test pulse to 10 s and maintaining a 10-s holding interval, the rate of channel block is slower, $\tau = 185 \pm 25$ s (mean ± S.E., 5 oocytes). The rate of block slows further when the 10-s test pulse was maintained, but the interpulse holding voltage interval is reduced to 3 s, $\tau = 262 \pm 32$ s (mean ± S.E., 5 oocytes). While the magnitude of block achieved in all 3 protocols is unchanged, the rate of block slows as the fraction of time spent at the test potential increases. This demonstrates that $MTSEA^+$ reacts more readily at voltages that favor the closed channel state and suggests a close association of MinK gating structures and the binding site for $MTSEA^+$ since its exposure changes as channels move between closed and open states. A similar state-dependent enhancement of binding site exposure is observed when Shaker K^+ channels carry a cysteine in their external pore region and move from open to an inactive conformation (33, 34).

The effect of voltage on block kinetics was evaluated by employing a constant duty cycle (3-s test pulse, 10-s holding interval) and comparing test pulse voltages of 0 and +40 mV with a holding voltage of −80 mV. The rate of $MTSEA^+$ block is slower at the more positive potential, $\tau = 160 \pm 14$ s (mean ± S.E., 4 oocytes), although the final magnitude of block is unchanged. This is consistent with the predicted effect of voltage on block by a positively charged agent that moves into the ion conduction pore and, thus, experiences the transmembrane electric field (4, 35). However, the effect of voltage may be a manifestation of state-dependent block and a slowly activating channel, since the more positive potential moves channels out of the $MTSEA^+$ reactive state more rapidly. Thus, despite this voltage dependence, the $MTSEA^+$ site may be superficial to the transmembrane electric field and the influence of voltage secondary to state-dependent exposure.

Block of C107A-MinK Current by $MTSEA^+$ Is Not Due to Changes in Channel Gating—While the kinetics of $MTSEA^+$ blockade are sensitive to channel state, modification does not lead to inhibition of current as a result of slowed channel opening or speeded channel closure (Table I). Although the activation of MinK channels are complex (36, 37), a qualitative approximation of activation kinetics can be achieved by comparing the amplitude of currents at 2 and 10 s while deactivation kinetics are well described by the sum of two exponentials (13). In fact, channels activate slightly more rapidly after $MTSEA^+$ modification than before and close slightly more slowly. If this were the only effect of $MTSEA^+$ on MinK, treatment would be expected to augment, rather than diminish, currents. This indicates that another mechanism underlies inhibition, perhaps direct pore occlusion, as seen with MTS reagents in other K^+ channels (31, 32).

$MTSEA^+$ Modification Alters the Fine Ionic Selectivity of C107A-MinK Channels—To test whether $MTSEA^+$ modification alters properties associated with the MinK pore, ion selectivity can be assessed by tail current reversal potential measurements (Fig. 3). Oocytes are held at −80 mV, pulsed to +20 mV to open the channels, and then repolarized to various test potentials; the initial slopes of tail currents are examined to determine reversal potential (V_{rev}). Unmodified C107A-MinK channels, like wild-type MinK (4), exhibit nearly ideal selectivity for K^+ over Na^+, showing a change of 55 ± 2 mV in V_{rev} with a 10-fold change in bath K^+ concentration (Table I), close to the 58-mV shift predicted by the Nernst relationship. On the other hand, $MTSEA^+$-modified channels shift their reversal potential only 38 ± 2 mV (Table I). This indicates that ion selectivity of modified channels has been altered.

To investigate how selectivity is altered, the fine ionic discrimination among monovalent K^+ ion analogs by unmodified and modified channels can be compared. Tail current reversal potentials are analyzed in terms of the Goldman-Hodgkin and Katz relation with each test ion as the only monovalent cation in the bath solution (Table II) (4). These "pseudo-biionic" conditions allow a comparison of the relative permeability of the

TABLE I

Effect of MTSEA on the ion selectivity, gating kinetics, and blocking parameters of C107A-MinK

Tail reversal potentials were determined by opening the channels with a 5-s pulse to +20 mV and measuring current 50 ms after shifting to test potentials from −10 to −110 mV in 20-mV steps. Activation kinetics were evaluated by calculating the ratio of macroscopic currents measured at 2 and 10 s elicited by a 10-s pulse to +20 mV from a holding potential of −80 mV. Deactivation was fit according to $I(t) = A_0 + A_1\exp(t/t_1) + A_2\exp(t/t_2)$ where A_0 represents the current at steady state, and A_1 and A_2 the amplitudes of the components represented by time constants t_1 and t_2, respectively (13). Inhibition constants (K_i) were determined after leak subtraction. Test solutions were ND-96 in which NaCl was isotonically substituted with 3 mM barium chloride (Ba^{2+}) or 75 mM TEA chloride. Voltage-dependent block was studied by opening the channels with a 10-s command pulse to +20 mV and measuring current 25 ms after repolarizing to test potentials between −50 and 10 mV. Electrical distance ($z\delta$) was calculated according to $K_i(V) = K_i(0)\exp(z\delta FV/RT)$, where $K_i(0)$ is the zero-voltage inhibition constant, z the valence of the blocking ion, and δ the fraction of applied voltage drop experienced at the blocker's binding site, as described (13). Values are mean ± S.E. for 5–8 oocytes.

	Before MTSEA	After MTSEA
Activation		
I_{10s}/I_{2s}	3.5 ± 0.3	4.7 ± 0.5
Deactivation (ms)		
t_1	400 ± 10	450 ± 70
t_2	2200 ± 200	2600 ± 300
ΔV_{rev}		
2–20 mM K^+	55 ± 2	38 ± 2
Ba^{2+}		
K_i, mM, 0 mV	3.5 ± 0.3	2.8 ± 0.4
TEA		
K, mM, 0 mV	145 ± 6	140 ± 10
$z\delta$	0.15 ± 0.02	0.16 ± 0.02

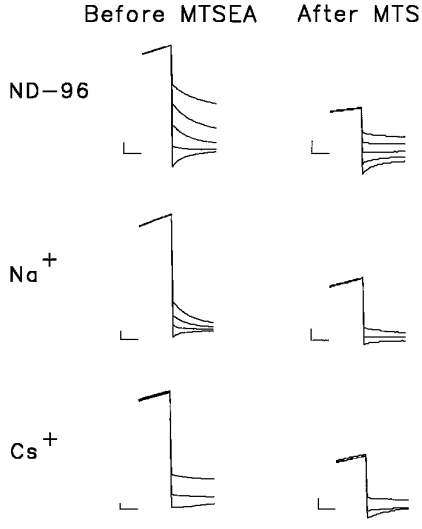

FIG. 3. **Reversal potentials of C107A-MinK before and after $MTSEA^+$ modification.** Tail current reversal potentials were measured before and after treatment of oocytes expressing C107A-MinK with 2.5 mM MTSEA for 10 min. Whole cell currents were elicited by a 5-s command pulse to +20 mV from a holding potential of −80 mV, then repolarized back various test potentials in the presence of the indicated solutions (see "Experimental Procedures"). The last 1 s of the command pulse and the first 1.5 s of the test pulse is shown in each trace. Conditions: *ND-96*, test potentials −30 to −110 mV in 20-mV steps before and after $MTSEA^+$; *Na^+* (98 mM Na^+ solution), test potentials −30 to −110 mV before and −50 to −90 mV after $MTSEA^+$ in 20-mV steps; *Cs^+* (98 mM Cs^+ solution), test potentials −40 to −80 mV in 20-mV steps before and after $MTSEA^+$. *Scale bars* are 100 nA and 0.5 s before and 50 nA and 0.5 s after $MTSEA^+$ treatment.

TABLE II
Effect of MTSEA on reversal potential and permeability ratios of C107A-MinK

Reversal potentials were measured under pseudo-biionic conditions, with 98 mM indicated cation in the external solution (see "Experimental Procedures") by the protocol of Fig. 3. Permeability ratios are calculated according to $P_K/P_X = \exp(-FV_{rev}/RT)$ where *R, T,* and *F* have their usual meanings, and P_K and P_X are the permeability of K^+ and test ion, respectively (4); this assumes K^+ is the only permeant ion inside the oocyte and, thus, underestimates the relative permeability of Na^+ through modified channels. Values are mean ± S.E. for 4–9 oocytes.

	K^+	Rb^+	Cs^+	Na^+	Li^+	$NMDG^+$
V_{rev} (mV)						
Before	1 ± 1	11 ± 2	−70 ± 2	−144 ± 6	<−150	<−150
After	1 ± 1	11 ± 2	−50 ± 2	−112 ± 8	<−150	<−150
P_k/P_x						
Before	1	1.5	15.4	280	>400	>400
After	1	1.6	7.3	110	>400	>400

test ion and K^+ (the predominant permeant ion inside the cell) before and after $MTSEA^+$ exposure. $MTSEA^+$ modification does not alter the relative permeability series for C107A-MinK channels ($K^+ > Rb^+ > Cs^+ > Na^+ \gg Li^+$, $NMDG^+$) or their ability to exclude Li^+ and $NMDG^+$ (Table II). However, modification does increase the relative permeability of channels for both Cs^+ and Na^+ by at least 2–3-fold (Fig. 3 and Table II).

An increase in Na^+ permeability through modified MinK channels should decrease the net outward current seen with depolarization since inward Na^+ flux will now offset outward K^+ current. This mechanism underlies the loss of K^+ currents in granule cells of *weaver* mice and results from a mutation in the pore of their GIRK2 K^+ channels (38, 39). However, increased Na^+ permeability is insufficient to explain the majority of the observed current reduction in $MTSEA^+$-modified MinK channels; permeability for K^+ remains 100-fold greater than for Na^+, and tail currents do not show augmented Na^+ conductance through modified channels (Fig. 3). Of note, increased Na^+ permeability does not develop in a linear relationship with the appearance of MinK channel blockade; no change in Na^+ permeability is seen until more than 70% of the current is inhibited when reversal potentials are measured in 98 mM Na^+ solution (n = 16 oocytes). That initial modification events lead only to channel blockade while later events result in changes in ion selectivity argues that more than one reactive cysteine is modified in each channel (on one or more channel subunits).

Permeability changes cannot be attributed to complete blockade of MinK channels and the exposure of less-selective ion channels endogenous to oocytes. Such endogenous currents are not seen in control cells. Moreover, residual unblocked currents exhibit characteristics of MinK channels including slow, voltage-dependent activation, rapid deactivation, sensitivity to barium, and voltage-dependent blockade by TEA (Table I). Partial blockade of MinK by $MTSEA^+$ is like the effect of this agent on other K^+ channels (31, 32, 34).

Block by TEA or $MTSES^-$ Does Not Interfere with $MTSEA^+$ Binding—Previously, we found that TEA binds in the external pore of the MinK channel, and its presence in the bath solution slows the rate of reaction of negatively charged $MTSES^-$ with a A45C/C107A-MinK, a mutant with a cysteine in position 45; this suggests the sites for TEA and $MTSES^-$ overlap (16). Conversely, TEA and $MTSEA^+$ do not appear to share overlapping binding sites since the rate of block of C107A-MinK by 2.5 mM $MTSEA^+$ is not altered by the presence of 75 mM TEA, τ = 111 ± 16 s (mean ± S.E., 5 oocytes). Consistent with this conclusion, the equilibrium inhibition constant (K_i) for TEA blockade of C107A-MinK is the same before and after $MTSEA^+$ modification (Table I). Sites for $MTSEA^+$ and $MTSES^-$ are also non-overlapping. After complete reaction of A45C/C107A-MinK channels with $MTSES^-$, the magnitude and rate of subsequent $MTSEA^+$ modification is unchanged, τ = 104 ± 9 s (mean ± S.E., 6 oocytes).

DISCUSSION

In the present study, we demonstrate that two sulfhydryl-specific reagents, $MTSEA^+$ and $MTSET^+$, alter the function of cysteine-free MinK channels in oocytes. The reagents react from the extracellular solution in a state-dependent fashion, modifying channels more rapidly when they are closed. Modification acts to change two attributes of MinK pore function. First, ion conduction is inhibited, apparently by a blocking mechanism since channel gating remains largely unchanged. Second, the channels' selectivity among monovalent cations is altered. That modification leads first to blockade and subsequently to changes in ion permeability suggests that more than one site is modified. Based on these results and other recent work (13, 16), we propose that MinK channels are formed through the intimate association of two MinK monomers and one or more copies of an oocyte membrane protein. Both MinK and the oocyte protein are exposed to the external solution, both change their local environment during channel gating, and both contribute to ion conduction pore function.

Negatively charged $MTSES^-$, while somewhat smaller than $MTSET^+$, has no effect on C107A-MinK. This suggests that the $MTSET^+$ site, if in the channel pore, is beyond the position that selects against permeation by negative ions. Like point mutation of residues in the pore-forming P domains of other K^+ channels (38–40), $MTSEA^+$ modification alters selectivity filter function. While it is tempting to speculate that modification occurs in proximity to the MinK channel selectivity filter (and, further, that the reactive site is near a P-like domain on the oocyte protein), it is important to emphasize that our results do not provide information about the mechanism by which ion selectivity is altered or show that modification has a local effect on ion permeation. While the findings are consistent with the idea that $MTSEA^+$ binds in the channel pore, such a conclusion cannot be made from studies of ion permeation alone. The findings do allow us to answer the question motivating this study, one or more copies of a cysteine-bearing oocyte protein are intimately associated with each MinK channel.

The wide diversity of K^+ channel functions observed *in vivo* reflect the many genes encoding K^+ channel subunits, production of splice variants, and the heteromultimeric association of channel subunits with distinctive properties (20). While some channels are functional as homomeric complexes, many are functional only when different subunit species co-assemble (41, 42). One such K^+ channel, GIRK1, whose natural partner is the cardiac inward rectifier subunit CIR (41), is able to function in oocytes only because it can assemble with a channel subunit endogenous to oocytes (XIR) to form functional channel complexes (43). MinK channels have been speculated to require co-assembly of MinK and another protein endogenous to oocytes, cardiocytes, or vestibular dark cells (13, 16, 22, 23). We show here that functional MinK channels are, indeed, heteromultimeric.

Note Added in Proof—Consistent with our findings and conclusions, two reports now indicate that MinK protein can form functional heteromultimeric channels with K_vLQT1, a single P domain K^+ channel subunit present in human heart (Sanguinetti, M. C., Curran, M. E., Zou, A., Shen, J., Spector, P. S., Atkinson, D. L., and Keating, M. T. (1996) *Nature* **384,** 80–83 and Barhanin, J., Lesage, F., Guillemare, E., Fink, M., Lazdunski, M., and Romey, G. (1996) *Nature* **384,** 78–80). Sanguinetti *et al.* also reveal the partial predicted sequence of a *Xenopus laevis* homolog (XK_vLQT1) which may prove to be the cysteine-bearing protein endogenous to oocytes whose association with MinK we study here.

REFERENCES

1. Takumi, T., Ohkubo, H., and Nakanishi, S. (1988) *Science* **242,** 1042–1045
2. Swanson, R., Hice, R. E., Folander, K., and Sanguinetti, M. C. (1993) *Sem. Neurosci.* **5,** 117–124
3. Hausdorff, S. F., Goldstein, S. A. N., Rushin, E. E., and Miller, C. (1991) *Biochemistry* **30,** 3341–3346
4. Goldstein, S. A. N., and Miller, C. (1991) *Neuron* **7,** 403–408
5. Folander, K., Smith, J. S., Antanavage, J., Bennett, C., Stein, R. B., and Swanson, R. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 2975–2979
6. Freeman, L. C., and Kass, R. S. (1993) *Circ. Res.* **73,** 968–973
7. Varnum, M. D., Busch, A. E., Bond, C. T., Maylie, J., and Adelman, J. P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 11528–11532
8. Marcus, D. C., and Shen, Z. (1994) *Am. J. Physiol.* **267,** C857–C864
9. Attali, B., Guillemare, E., Lesage, F., Honore, E., Romey, G., Lazdunski, M., and Barhanin, J. (1993) *Nature* **365,** 850–852
10. Shimbo, K., Brassard, D. L., Lamb, R. A., and Pinto, L. H. (1995) *Biophys. J.* **69,** 1819–1829
11. Tzounopoulos, T., Maylie, J., and Adelman, J. (1995) *Biophys. J.* **69,** 904–908
12. Takumi, T., Moriyoshi, K., Aramori, I., Ishii, T., Oiki, S., Okada, Y., Ohkubo, H., and Nakanishi, S. (1991) *J. Biol. Chem.* **266,** 22192–22198
13. Wang, K. W., and Goldstein, S. A. N. (1995) *Neuron* **14,** 1303–1309
14. Busch, A. E., Varnum, M. D., North, R. A., and Adelman, J. P. (1992) *Science* **255,** 1705–1707
15. Zhang, Z. J., Jurkiewicz, N. K., Folander, K., Lazarides, E., Salata, J. J., and Swanson, R. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 1766–1770
16. Wang, K. W., Tai, K. K., and Goldstein, S. A. N. (1996) *Neuron* **16,** 571–577
17. Tzounopoulos, T., Guy, H. R., Durell, S., Adelman, J. P., and Maylie, J. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92,** 9593–9597
18. Ketchum, K. A., Joiner, W. J., Sellers, A. J., Kaczmarek, L. K., and Goldstein, S. A. N. (1995) *Nature* **376,** 690–695
19. Goldstein, S. A. N., Price, L. A., Rosenthal, D. N., and Pausch, M. H. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93,** 13256–13261
20. Hille, B. (1992) *Ionic Channels of Excitable Membranes,* 2nd Ed., Sinauer, Sunderland, MA
21. MacKinnon, R. (1995) *Neuron* **14,** 889–892
22. Blumenthal, E. M., and Kaczmarek, L. K. (1994) *J. Neurosci.* **14,** 3097–3105
23. Lesage, F., Attali, B., Lakey, J., Honore, E., Romey, G., Faurobert, E., Lazdunski, M., and Barhanin, J. (1993) *Recept. Channels* **1,** 143–152
24. Yang, T., Kupershmidt, S., and Roden, D. M. (1995) *Circ. Res.* **77,** 1246–1253
25. Stauffer, D. A., and Karlin, A. (1994) *Biochemistry* **33,** 6840–6849
26. Akabas, M. H., Stauffer, D. A., Xu, M., and Karlin, A. (1992) *Science* **258,** 307–310
27. Goldstein, S. A. N. (1996) *Neuron* **16,** 717–722
28. Smith, D. J., Maggio, E. T., and Kenyon, G. L. (1975) *Biochemistry* **14,** 766–771
29. Akabas, M. H., Kaufmann, C., Cook, T. A., and Archdeacon, P. (1994) *J. Biol. Chem.* **269,** 14865–14868
30. Akabas, M. H., and Karlin, A. (1995) *Biochemistry* **34,** 12496–12500
31. Kurz, L. L., Zuhlke, R. D., Zhang, H. J., and Joho, R. H. (1995) *Biophys. J.* **68,** 900–905
32. Pascual, J. M., Shieh, C. C., Kirsch, G. E., and Brown, A. M. (1995) *Neuron* **14,** 1055–1063
33. Yellen, G., Sodickson, D., Chen, T. Y., and Jurman, M. E. (1994) *Biophys. J.* **66,** 1068–1075
34. Liu, Y., Jurman, M. E., and Yellen, G. (1996) *Neuron* **16,** 859–867
35. Woodhull, A. M. (1973) *J. Gen. Physiol.* **61,** 687–708
36. Hice, R. E., Folander, K., Salata, J. J., Smith, J. S., Sanguinetti, M. C., and Swanson, R. (1994) *Pfluegers Arch. Eur. J. Physiol.* **426,** 139–145
37. Cui, J., Kline, R. P., Pennefather, P., and Cohen, I. S. (1994) *J. Gen. Physiol.* **104,** 87–105
38. Kofuji, P., Hofer, M., Millen, K. J., Millonig, J. H., Davidson, N., Lester, H. A., and Hatten, M. E. (1996) *Neuron* **16,** 941–952
39. Navarro, B., Kennedy, M. E., Velimirovic, B., Bhat, D., Peterson, A. S., and Clapham, D. E. (1996) *Science* **272,** 1950–1953
40. Heginbotham, L., Lu, Z., Abramson, T., and MacKinnon, R. (1994) *Biophys. J.* **66,** 1061–1067
41. Krapivinsky, G., Gordon, E. A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D. E. (1995) *Nature* **374,** 135–141
42. Inagaki, N., Gonoi, T., Clement, J. P., IV, Namba, N., Inazawa, J., Gonzalez, G., Aguilar-Bryan, L., Seino, S., and Bryan, J. (1995) *Science* **270,** 1160–1170
43. Hedin, K. E., Lim, N. F., and Clapham, D. E. (1996) *Neuron* **16,** 423–429